A NEW MEDIUM FOR THE DIFFERENTIATION OF *B. COLI* IN WATER ANALYSIS

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During the course of an investigation into the bacteriology of the water supplies of the colony the repeated isolation of *B. coli* (lactose +, indol +) and of Houston's atypical *B. coli* from small quantities (1 c.c., ·1 c.c., and ·01 c.c.) of raw natural waters which were declared on sanitary survey to be free from all possibility of human faecal pollution and very remotely, if at all, liable to any animal contamination, centred attention on the sufficiency of the methods for the bacterioscopic examination of waters recommended by a Committee of the Royal Institute of Public Health in 1903, and adopted by bacteriologists in England and elsewhere as the standard method, and the interpretation of the results obtained by this method.

The literature of the bacteriology of tropical waters reveals frequent references to the presence of *B. coli* as detected by the standard method in waters which would be certified as pure and free from objectionable pollution by the comparative freedom from water-borne diseases among those drinking such waters, and by the results of sanitary surveys.

Daniels (1908), working on the waters from jungle streams of the Federated Malay States, observed that it was exceptional to find a jungle stream from which *B. coli* could not be isolated in 2 c.c., even though such waters would appear to be free from human and animal faecal pollution. Archibald (1910), commenting on the waters of the Soudan, remarked :

> ' If samples of water taken from shallow wells or rivers in the Tropics are subjected to a few simple tests for the presence of faecal contamination, the results will often show such a state of things that no analyst in England would ever consider the passing of such waters as fit for human consumption, and yet the water from those sources is used daily by both Europeans and natives alike without any ill effects to health as far as can be told. The question naturally arises whether in the face of these existing conditions one would be justified in using European standards of water purity as a guide or whether some modification of the European standard could be generally employed in tropical climes ? '

Wise and Minett (1912) isolated *B. coli* in from 1 to ·001 c.c. of raw waters from various sources used for drinking purposes by the inhabitants of British Guiana. Clemesha in India (1908-1912), struck by the high degree of 'faecal' (?) pollution in the drinking water supplies of the Madras Presidency as evidenced by the presence of *B. coli* isolated by the standard method, conducted a series of interesting experiments demonstrating the value of sunlight in the process of purification of the waters of India.

B. coli as defined by the standard method, and by Houston, embraces more than ten varieties of organisms, and though one or more varieties may be present in a water supply, the natural purification which that water undergoes from exposure to sunlight destroys those organisms which are objectionable or are evidence of objectionable pollution.

Hence the mere statement by a water analyst of the isolation of *B. coli* in a certain quantity of water is, as regards the Tropics, at all events, of comparatively little value from the sanitary point of view unless the effects of self-purification are taken into account. If, he says, the standard method be applied in its entirety to India, nearly every drop of drinking water in that country would be condemned.

In England the raw waters of the Thames investigated by Houston are waters to which sewage and other pollution gain constant access and *B. coli* in such waters would almost invariably be of faecal origin. Unfortunately the literature available locally does not record the result of quantitative examinations for the presence of *B. coli* in the raw natural waters of England, waters obtained from uninhabited regions and not exposed to human or animal faecal pollution.

Thresh, however, refers to a public water supply from the Welsh moorlands *in which no sewage contamination* was possible, but in which *B. coli* (lactose +, indol +) was present in 1 c.c. Such a water, he recommends, should be filtered before being delivered to the consumer. Does this example, though solitary, indicate that *B. coli* may perhaps be present in raw natural waters not exposed to human or animal pollution in temperate regions as is the case in the Tropics?

The standard method postulates that all *B. coli* are of faecal

origin without regard to the fact that such organisms are frequently found not only in small quantities of raw natural waters free from all faecal pollution, but also in *unpolluted soil*. From the soil free from faecal contamination these organisms may gain entrance to water supplies, and such supplies may be condemned by the standard method as polluted and unfit for human consumption. Chen and Rettger (1919) found 156 out of 467 (33·4 per cent.) coli-like organisms isolated from unpolluted soil to be lactose +, indol + organisms and Max Levine 37·3 per cent. out of 177 lactose fermenters of the soil to be also indol producers.

In Trinidad, of 120 cultures isolated from unpolluted soil by the standard method 42 per cent. were typical *B. coli* (lactose +, indol +) and if Houston's atypical *B. coli* be included, the percentage of *B. coli* regarded as an index of faecal pollution would be considerably higher.

If lactose +, indol + *B. coli* may be isolated by the standard method from soils to which no faecal matter has gained entrance, is a method which does not attempt to differentiate between faecal and non-faecal *B. coli* sufficient to justify an analyst in expressing an opinion upon the sanitary quality of a water, particularly if the sanitary control of that water is liable to variation ? At all events, should the water analyst not attempt to indicate the faecal or non-faecal origin of *B. coli* isolated from water ?

Keyes, Rogers, Clarke and others (1909-1914) showed by accurate determination of the gas volumes and gas ratios produced in the anaerobic fermentations of glucose that the non-spore-bearing lactose fermenters of faeces can be divided into two groups, one a low ratio or *B. coli* group in which the proportion of CO_2 to H_2 is almost constantly equal to 1·06 and the other a high ratio or B. aerogenes-cloacae group which produces considerably more CO_2 than H_2, with a wide range of ratio between these gases.

Clark and Lubs (1915) showed that in a carefully adjusted sugar medium the low ratio organisms produce a relatively high hydrogen ion concentration which can be recognised by an indicator, such as methyl red becoming red, whilst the high ratio organisms produce a low hydrogen ion concentration and methyl red becomes yellow.

In human faeces, according to Rogers, Clarke and Lubs, the low ratio group (methyl red +, Voges-Proskauer −) constitutes

74 per cent., and the high ratio group 26 per cent., of the lactose fermenters, whilst in bovine faeces the low ratio group constitutes 99·4 per cent., and the high ratio group ·6 per cent. (Rogers).

Chen and Rettger, in 1919, found all of 173 organisms from faeces to be methyl red positive and Voges-Proskauer negative.

In Trinidad, of 740 cultures isolated from human, bovine, and equine faeces, 94 per cent. were methyl red positive and all Voges-Proskauer negative.

On the other hand, Chen and Rettger found that of 467 coli-like organisms isolated from unpolluted soils, 430 belonged to the high ratio (methyl red —, V.-P. +) or B. aerogenes-cloacae group, and 20 to the low ratio *B. coli* group and, as stated above, 33·4 per cent. of these 467 were lactose +, indol +.

In Trinidad, of 120 cultures isolated from unpolluted soil, 85 per cent. were methyl red negative and 15 per cent. methyl red positive, and as previously pointed out 42 per cent. were lactose +, indol +.

But the gas ratio determination is not possible in the ordinary laboratory analysis of water, and whilst the methyl red and Voges-Proskauer tests have been found in the case of faeces and soil to indicate fairly accurately the habitat of the organism under investigation, their application in the bacteriological analysis of waters has been shown to afford no clue as to the source (faeces or soil) of the organism isolated from a water. Thus Winslow and Cohen found the percentage of methyl red positive, Voges-Proskauer negative organisms to be practically the same in polluted, unpolluted and stored raw waters. Out of 255 coli-like organisms, 76 per cent. from unpolluted, 77 per cent. from polluted and 85 per cent. from stored rain water were methyl red positive and V.-P. negative. Stewart Koser found 80·4 per cent. of the colon group cultures obtained from polluted waters and 73·3 per cent. from unpolluted waters to be methyl red positive and Voges-Proskauer negative.

In Trinidad, of 220 organisms isolated from polluted waters, 87·3 per cent. were methyl red positive, 6·3 per cent. methyl red negative, and 6·4 per cent. doubtful; and of 240 cultures obtained from sanitarily pure waters 42·5 per cent. were methyl red positive and 57·5 per cent. negative. Though the American Public Health Association (1923 ed.) recommends the methyl red and Voges-Proskauer tests in the bacteriological examination of water, local

experience supports the conclusion of Winslow, Cohen, Stewart Koser and others that the lack of correlation between these tests and the sanitary qualities of waters justifies little reliance being placed upon them as indices of sanitary purity.

Stewart Koser (1923), in a study of the utilisation of salts of various organic acids, found that the two sections of the colon group of organisms could be clearly distinguished by the use of a chemically definite medium containing sodium, potassium or ammonium citrate as the only source of carbon. Such a synthetic medium can be made by dissolving 1·5 grammes microcosmic salt Na (NH_4) PO_4 + H_2O, 1 gramme KH_2 PO_4, 0·2 gramme $MgSO_4$ and 2 grammes sodium citrate in 1000 c.c. distilled water, tubing, and autoclaving at 120° C. for fifteen minutes. A clear colourless liquid is obtained. In this medium Stewart Koser found that 90·7 per cent. of *B. coli* isolated from faeces failed to develop, whilst the B. aerogenes-cloacae group produced a visible turbidity within forty-eight hours at 30° C. This differentiation correlated with the methyl red and Voges-Proskauer tests as far as the typical *B. coli* type and the aerogenes section in faeces are concerned.

With regard, however, to organisms isolated from the soil, he found that a number were consistently methyl red positive and Voges-Proskauer negative, although they had been obtained from soils regarded as free from pollution. When tested in the citrate medium these soil coli were found to utilise it. Of 72 cultures obtained from unpolluted soils, 97·2 per cent. utilised the citrate with the production of a visible turbidity and were distinct from faecal *B. coli*, whilst the methyl red test showed 51·4 per cent. alkaline to methyl red and the Voges-Proskauer 52·8 per cent. positive.

In Trinidad, of 432 cultures isolated from human, bovine, and equine faeces, 96·3 per cent. failed to develop in the citrate medium, while 3·7 per cent. did so; and in the case of unpolluted soils, of 214 cultures of the coli group, 90 per cent. utilised the citrate medium in forty-eight hours and 10 per cent. failed to do so. The citrate medium as a biological test is thus an accurate indicator of the habitat of the coli organism isolated from faeces and soil. In the application of the citrate medium for the differentiation of faecal and non-faecal *B. coli* obtained from waters, very striking results have been obtained. Samples of waters were secured from localities

in Trinidad where the chances of human intestinal pollution were impossible and contamination by birds and an occasional wild animal practically negligible ; by the standard method, *B. coli* were isolated and put through the citrate, methyl red and Voges-Proskauer tests. Of 240 cultures thus obtained, 81·3 per cent. grew in the citrate medium and 18·7 per cent. failed to do so; whilst of 210 *B. coli* isolated at various periods from polluted streams below villages 90·9 per cent. failed to utilise the citrate and 9·1 per cent. produced a distinct turbidity. The citrate utilisation by *B. coli* is thus seen to afford some degree of correlation with the sanitary survey of a water supply. Further, the *B. coli* colonies (lactose +, indol +) isolated from a certain quantity of water, say 1 c.c., by the standard method, may, by the citrate test, be shown to be of non-faecal origin and it is only in a larger quantity of water (5·10 or 25 c.c.) that faecal (citrate —) *B. coli* (lactose +, indol +) is found. Whilst, therefore, by the routine standard method a water may be condemned, by the use of the citrate test the bacteriological analysis of a water supply may be found to harmonise with epidemiological and sanitary conditions. To those, therefore, engaged in water analysis in the Tropics, particularly of those waters where that perfect sanitary control obtained by Sir Alexander Houston for the waters of the Metropolitan Water Board can only be an impossible vision, Stewart Koser's remarks should be of special interest. He says :

' the primary results shewn by the citrate medium indicate that this method of differentiation is deserving of further study with regard to its usefulness and application in the sanitary examination of water supplies, though the final acceptance of any such test must of course await general confirmation at the hands of different workers.'

Such a test is necessary. For is the relatively low incidence, in certain parts of the Tropics, of water-borne diseases, in contrast with the high degree of faecal pollution as evidenced by the presence of *B. coli,* detected by the standard method, due to the constant accidental absence of the specific pathogenic organisms or to the natural purification which waters in the Tropics undergo from exposure to sunlight in addition to the fact that by the standard method no attempt is made to differentiate between faecal and non-faecal *B. coli* ?

SUMMARY

1. *B. coli* (lactose +, indol +) may be isolated by the standard method not only from faeces and polluted waters, but also from unpolluted soils and unpolluted waters.

2. As a standard indicator of faecal contamination its value is not therefore unquestionable.

3. Local experience indicates that the utilisation of citrate by *B. coli* may be of value in differentiating faecal from non-faecal *B. coli* in water analysis.

UNPOLLUTED SOIL

	In Trinidad		In America (Chen and Rettger)		In America (Levine)	
		No. of Colonies studied		No. of Colonies studied		No. of Colonies studied
Lactose + Indol + *B. coli*	42 per cent.	214	33·4 per cent.	467	37·3 per cent.	177

CITRATE TEST IN AMERICA (STEWART KOSER)

	Growth in Citrate	No growth in Citrate	No. of Colonies studied
Faeces	9·3 per cent.	90·7 per cent.	118
Unpolluted Soil ...	97·2 per cent.	2·8 per cent.	72

CITRATE TEST IN TRINIDAD

	Growth in Citrate	No growth in Citrate	No. of Colonies studied
Faeces	3·7 per cent	96·3 per cent.	432
Polluted Water ...	9·1 per cent.	90·9 per cent.	210
Unpolluted Soil ...	90 per cent.	10 per cent.	214
Unpolluted Water ...	81·3 per cent.	18·7 per cent.	240

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